Applicants' Invention

Applicants' claimed invention is drawn to methods for genetically modifying human hematopoietic stem cells (HSC) and methods for transducing human CD34+ hematopoietic cells. The methods comprise contacting a vector comprising a polynucleotide sequence encoding a heterologous gene with a population of human HSC, or with a population of mammalian $CD34^+$ hematopoietic cells that includes a subpopulation of human HSC, where the human HSC or human CD34+ hematopoietic cells are cultured under the recited culture conditions. In each method, the cells are cultured in the presence of an mpl ligand and a flt3 ligand, both of which are present in the culture system at a concentration of about 0.1 ng/mL to about 500 ng/mL. In alternative embodiments, the HSC or CD34+ hematopoietic cells are cultured in the presence of additional cytokines, including c-kit ligand, IL-3, IL-6, and/or LIF at the recited effective amounts.

The technical challenge facing genetic manipulation of hematopoietic stem cells is one of triggering HSC to enter the cell cycling phase to allow for integration of genetic material of interest and an increase in the amount of genetically modified HSC for subsequent transplantation, while keeping the genetically modified HSC in a pluripotent state so that long-term engraftment can successfully provide a continuous supply of genetically modified hematopoietic cells during the life of a patient. The methods of the present invention provide a means for addressing this technical challenge. First, the claimed culture conditions produce a subpopulation of actively cycling HSC, which are critical for some retroviral (i.e., non-lentiviral-based) infection and nucleic acid integration of heterologous genetic material of interest. Second, active cycling of this subpopulation of HSC generates increases in the number of HSC available for genetic manipulation and subsequent transplantation. Third, these actively cycling HSC retain their multi-lineage pluripotency, that is they retain their self-renewal capacity as well as their ability to give rise to all other hematopoietic cell lineages. For purposes of the discussion herein below, such HSC will be referred to as multi-lineage pluripotent HSC. Further, these three characteristics can be accomplished in the absence of stroma in the cell culture system that is present during the time period for retroviral infection.

The particular culture conditions recited in the claims provide for proliferation of human pluripotent HSC that are capable of supporting long-term engraftment as demonstrated by the SCID-hu bone assay for repopulating activity, and which can be effectively transduced to achieve genetic modification of these pluripotent HSC. See the data presented in Example 5 and Examples 8 and 9, respectively. In a technical field that has proven to be highly unpredictable, Applicants are the first to demonstrate the advantages of including a combination of mpl ligand and flt3 ligand with or without additional cytokines in the culture system for HSC targeted for genetic manipulation and clinical protocols aimed at long-term engraftment.

The Rejection of the Claims Under 35 U.S.C. §103(a), Should Be Withdrawn

The Office Action has indicated that claims 18-20, 23-27, 31-35, 37-44, and 46-47 stand rejected under 35 U.S.C. §103(a) in light of the following 18 references, which the Office Action has divided into 6 groups based on their teachings: Murray *et al.* (U.S. Patent 5,665,557), Nakahata *et al.* (U.S. Patent No. 5,861,315), Hoffman *et al.* (U.S. Patent No. 5,744,361), Fei *et al.* (U.S. Patent No. 5,635,387), and Davis *et al.* (U.S. Patent No. 5,599,703) (Group I); Ku *et al.* (1996), Ohmizono *et al.* (1996), Kobayashi *et al.* (1997), and Ramsfjell *et al.* (1997) (Group II);. Escary *et al.* (1993) and Szilvassy *et al* (1996) (Group III); Bodine *et al.* 1992) (Group IV); Tushinski *et al.* (Abstract), Fletcher *et al.* (1991), Bello-Fernandez *et al.* (1997), and Hatzfeld *et al.* (1996) (Group V); and, Hanenberg *et al.* (1996) and Hanenberg *et al.* (1997) (Group VI). Specifically the Office Action states that each of these 6 groups of references teaches one aspect of the claimed invention, and that the skilled artisan would be motivated to combine the references from these 6 groups to obtain the claimed invention as a whole, thereby rendering the claimed invention obvious. As discussed above, in the present application claims 48-51 are also pending. Therefore, the rejection will be traversed below as it applies to claims 18-20, 23-27, 31-35, 37-44, 46-47, and 48-51.

Applicants note at the outset that both the burden of proof and the evidentiary standard for determining obviousness are precisely defined. Specifically, the Examiner bears the burden of presenting a *prima facie* case for obviousness, with a showing of such *prima facie*

obviousness requiring: 1) some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings; 2) the teaching or suggestion of all the claim limitations of the Applicant's invention in the combined prior art references; and, 3) a reasonable expectation of success. *Manual of Patent Examining Procedure* (MPEP) §2143.

With regard to the first of these factors, suggestion or motivation to combine, such motivation may be found "where there is some teaching, suggestion, or motivation ... either explicitly or implicitly in the references themselves or in the knowledge generally available to one of ordinary skill in the art." MPEP §2143.01 (citing *In re Kotzab*, 217 F.3d 1365, 1370 55 USPQ2d 1313, 1317 (Fed. Cir. 2000)). Not only must such motivation be present, it must be *specific*. The Federal Circuit recently re-emphasized the importance of specific motivation to combine references, stating:

> When patentability turns on the question of obviousness, the search for and analysis of the prior art includes evidence relevant to the finding of whether there is a teaching, motivation, or suggestion to select and combine the references relied on as evidence of obviousness. *See, e.g., McGinley v. Franklin Sports, Inc.*, 262 F.3d 1339, 1351-52, 60 USPQ2d 1001, 1008 (Fed. Cir. 2001) ("the central question is whether there is reason to combine [the] references," a question of fact drawing on the Graham factors).
>
> "The factual inquiry whether to combine references must be thorough and searching." *Id.* It must be based on objective evidence of record. This precedent has been reinforced in myriad decisions, and cannot be dispensed with. *See, e.g., Brown & Williamson Tobacco Corp. v. Philip Morris Inc.*, 229 F.3d 1120, 1124-25, 56 USPQ2d 1456, 1459 (Fed. Cir. 2000) ("a showing of a suggestion, teaching, or motivation to combine the prior art references is an 'essential component of an obviousness holding'") (*quoting C.R. Bard, Inc., v. M3 Systems, Inc.*, 157 F.3d 1340, 1352, 48 USPQ2d 1225, 1232 (Fed. Cir. 1998)); *In re Dembiczak*, 175 F.3d 994, 999, 50 USPQ2d 1614, 1617 (Fed. Cir. 1999) ("Our case law makes clear that the best defense against the subtle but powerful attraction of a hindsight-based obviousness analysis is rigorous application of the requirement for a showing of the teaching or motivation to combine prior art references."); *In re Dance*, 160 F.3d 1339, 1343, 48 USPQ2d 1635, 1637 (Fed. Cir. 1998) (there must be some motivation, suggestion, or teaching of the desirability of making the specific combination that was made by the applicant); *In re Fine*, 837 F.2d 1071, 1075, 5 USPQ2d 1596, 1600 (Fed. Cir. 1988)

> ("'teachings of references can be combined only if there is some suggestion or incentive to do so.'") (emphasis in original) (*quoting ACS Hosp. Sys., Inc. v. Montefiore Hosp.*, 732 F.2d 1572, 1577, 221 USPQ 929, 933 (Fed. Cir. 1984)).

*In re Lee*, 61 U.S.P.Q.2d 1430, 1433-4 (Fed. Cir. 2002) (vacating and remanding the decision of the Board for failing to follow the relevant precedent).

Thus, the Examiner can satisfy the burden of obviousness of the combination only by showing either "some objective teaching in the prior art" or, alternatively, that there is "knowledge generally available to one of ordinary skill in the art [that] would lead that individual to combine the relevant teachings of the references." *Id.* at 1434 (citing *In re Fritch*, 972 F.2d 1260, 1265, 23 USPQ2d 1780, 1783 (Fed. Cir. 1992)).

In the instant case, a review of the prosecution history shows that the Examiner provides 18 references and concludes that, since each of the aspects of the invention is taught in one of the 6 groups into which these 18 references have been divided, the skilled artisan would be sufficiently motivated to combine the appropriate references so as to render the present invention obvious. The Examiner cites references teaching: methods of isolating and culturing populations of human hematopoietic stem cells (Group I); the effects of TPO (mpl-ligand) and flt-ligand (FL) on human hematopoietic stem cells in culture (Group II); the effects of LIF on hematopoietic stem cells in culture (Group III); the effects of SCF (c-kit ligand) and IL-6 on hematopoietic stem cells in culture (Group IV); methods of retroviral mediated gene transfer into hematopoietic stem cells in culture (Group V); and, the use of fibronectin to increase cell transformation of retroviral vectors (Group VI). See, e.g., the Office Action of April 10, 2000 (Paper 9), at pages 3-5. The Examiner then states that, in essence, *because these teachings were available in print at the time of the present invention*, it would have been *prima facie* obvious to one of ordinary skill in the art to combine them to obtain both the specific combinations of factors and concentrations of factors of the present invention. Thus, the Examiner asserts that the motivation to combine the provided references is evidenced by the fact that:

> [o]ne of ordinary skill in the art was aware at the time of the instant invention of the motivation to use those cytokines taught in the art on different populations of HSC in different cytokine concentrations as taught such that slightly different

> types of cells were tested with different concentrations of growth factors to optimize the growth (each reference cited teaches unique circumstances to their cell population).

Office Action mailed January 4, 2001 (Paper 12), at page 5. For example, with regard to the sets of factors used in the present invention, the Examiner has stated that:

> [i]t would have been prima facie obvious to one of ordinary skill in the art to culture human hematopoietic stem cells in mpl-ligand, flt3 ligand, c-kit-ligand, IL3, LIF, TPO, and/or IL-6 since [the references of Groups I-III] all teach methods for isolation and culturing of hematopoietic stem cells via addition of one or more of mpl-ligand, flt3 ligand, c-kit-ligand, IL3, LIF, TPO, and/or IL-6.

Paper 9, at pages 4-5. Thus, the Examiner concludes,

> ... as broadly claimed, the combination of the cited references provides one of ordinary skill in the art with the requisite motivation and expectation of success to make and use the invention as claimed, i.e. it was obvious to one of ordinary skill in the art to use the claimed combinations of factors as claimed.

Advisory Action (Paper 18), at page 2.

Applicants respectfully disagree with the Examiner and maintain that the Examiner has not met the burden of establishing a *prima facie* case of obviousness as the requisite showing of a motivation to combine these 18 references has not been demonstrated for reasons already made of record and further in view of the following remarks. Applicants take this opportunity to remind the Examiner that the pending claims are directed to methods for genetically modifying human hematopoietic stems cells (HSC) or human $CD34^+$ hematopoietic cells comprising a subpopulation of HSC.

The Examiner is in agreement that no one reference teaches or suggests Applicants' claimed invention, as the Examiner has relied upon 6 groups of references in support of this obviousness rejection. Thus, the issue at hand is whether the motivation to combine the cited references to provide the missing elements of Applicants' claimed invention not taught in a single cited reference is extant in the cited references or resided within the knowledge of the skilled artisan *at the time of Applicants' invention*. Applicants respectfully submit that a

thorough review of these 18 references reveals that either the motivation to combine the appropriate references is clearly absent or, where a suggestion or motivation may have existed, there was no reasonable expectation for success given the well-established unpredictability of genetic manipulation of pluripotent human HSC at the time of Applicants' invention.

*The Group V and VI References Directed to Transduction of HSC Provide No Motivation to Combine the Cited References.*

As genetic modification of pluripotent HSC is the key objective of Applicants' invention, Applicants first look to the cited references in this field, i.e., the references of Group V and Group VI, for a suggestion of the presently claimed invention, or a showing of motivation to combine any or all of these references to arrive at Applicants' claimed invention.

Group V References.

The first of the Group V references, the abstract by Tushinski *et al.*, is directed to retroviral transduction of human HSC isolated from mobilized peripheral blood (MPB) of individuals with multiple myeloma or breast cancer. This abstract teaches successful transduction of HSC cultured in interleukin-3 (IL-3) (20 ng/mL), IL-6 (20 ng/mL), and 100 ng/mL of either stem cell factor (SCF), leukemia inhibitory factor (LIF), or fetal liver kinase-2 receptor ligand (flt3 ligand, FL). The Tushinski *et al.* abstract provides no indication that modification of the culture conditions is necessary or desirable, as successful transduction HSC was accomplished. Further, the Tushinski *et al.* abstract fails to teach or suggest the use of an mpl ligand in combination with a flt3 ligand as part of the culture system during the step of transduction. Hence, Applicants submit that there was no motivation to modify this reference, or to combine the teachings of this reference with the teachings of the other references cited by the Examiner, to arrive at Applicants' claimed invention.

The second of the Group V references, Fletcher *et al.* (1991), teaches improved survival and improved overall recovery of retroviral vector-infected murine hematopoietic stem cells during short-term co-culture (72 hours) of murine bone marrow with retroviral vector-producing

fibroblasts when LIF (1000 units/mL) is included in the culture medium. No mention is made of other cytokines or of an mpl ligand or flt3 ligand in this culture medium. See the reference at page 838, second column, where the infection protocol is described. Applicants first note that the methods of transducing and culturing of murine hematopoietic stem cells do not necessarily provide for successful transduction and culture of human hematopoietic stem cells due to differences in the *in vivo* environmental requirements for murine and human pluripotent HSC function. That being said, this cited reference further teaches that infection efficiency of murine HSC recovered from these cultures was about 80%, which did not differ from infection efficiency of the control cultures in the absence of LIF, the greater overall recovery of infected stem cells being attributed to the much greater survival of the cultured stem cells in the presence of LIF. Finally, Fletcher *et al.* teach stable engraftment with the progeny of provirus-bearing stem cells in irradiated murine recipients of the LIF-treated marrow. Thus, this reference teaches a successful method for transduction of murine hematopoietic stem cells and their successful engraftment in murine recipients, and suggests that studies of LIF in human hematopoietic precursor cultures should indicate whether similar protocols will allow efficient transduction of human stem cells for the purpose of gene therapy. See the reference at page 44, last paragraph. Thus, at most this reference provides motivation for one of skill in the art to try a similar protocol for transduction of human HSC. It provides no motivation for one of skill in the art to modify the teachings of this reference, or to look to the other 17 cited references for ways to modify the teachings of this reference, to arrive at Applicants' claimed invention.

The third of the Group V references, Bello-Fernandez *et al.* (1997), teaches successful retrovirus-mediated gene transfer of dendritic cells under serum-free conditions with the use of a dendritic cell differentiating-inducing cytokine cocktail. Applicants first note that though the starting population of cells for this protocol is a population of $CD34^+$ cord blood cells, these isolated cells are cultured for 3 days in a manner to provide for differentiation of these cells into dendritic cell progenitors, which are then subjected to a transduction protocol. Thus, this reference teaches a transduction protocol that is relevant for transduction of a population of progenitor cells that have lost their multi-lineage pluripotent function. In contrast, the methods

of Applicants' claimed invention are directed to genetic modification of multi-lineage pluripotent HSC. That being said, the cytokine cocktail taught by Bello-Fernandez *et al.* includes TGF-$\beta$1, rhTNF$\alpha$, rhGM-CSF, rhSCF (20 ng/mL), and flt3 ligand (100 ng/mL). The Bello-Fernandez *et al.* reference fails to teach or suggest the use of an mpl ligand, i.e., TPO, in their cytokine cocktail, as the objective of the method taught by this reference is to obtain a population of progenitor stem cells that are committed to mature into dendritic cells. In contrast, Applicants' claimed invention requires the use of an mpl ligand, i.e., TPO, to be present in the culture system during transduction, as the objective of Applicants' invention is to achieve maximum proliferation of multi-lineage pluripotent HSC without committing these pluripotent HSC into specific pathways of lineage differentiation. Where the objectives of a cited reference are counter to the objectives of Applicants' invention, there can be no motivation to modify the cited reference to obtain Applicants' invention. In view of this, Applicants submit that there was no motivation to modify this reference, or to combine the teachings of this reference with the teachings of the other 17 cited references, to arrive at Applicants' claimed invention.

The fourth of the Group V references, Hatzfeld *et al.* (1996), teaches successful stable retroviral-mediated gene transfer into human umbilical cord $CD34^+$ HSC that have been pre-stimulated for 10 hours in a culture medium that includes a cytokine cocktail to release the HSC from their quiescent state. The cytokine cocktail includes IL-3 (1.7 units/ml), IL-6 (10 units/mL), GM-CSF (18 units/mL), steel factor (SF; 6 ng/mL), and anti-TGF-$\beta$ serum. The anti-TGF-$\beta$ serum, which is added to block the inhibitory effects of contaminating or autocrine TGF-$\beta$ on HSC cell cycling, augments the gene transfer efficiency that is achieved with IL-3, IL-6, GM-CSF, and steel factor. The reference further teaches that longer periods of pre-stimulation (20 or 48 hours) abrogate the beneficial effects of anti-TGF-$\beta$ serum on gene transfer efficiency. Hatzfeld *et al.* conclude that blocking of TGF-$\beta$ using antisense or anti-TGF-$\beta$ serum is necessary to rapidly release the stem cell compartment from quiescence and get efficient stable transduction using a retroviral system. See the Abstract, and page 210, first paragraph of the Discussion section. Hatzfeld *et al.* do not teach the use of an mpl ligand in combination with a flt3 ligand as set forth in Applicants' claimed invention. Further, where successful retroviral

transduction is taught, there is no motivation to modify this reference or to look to the other 17 cited references for guidance as to other modifications that should be carried out to arrive at Applicants claimed invention.

Group VI References.

The Office Action relies on the Group VI references to provide the motivation to include fibronectin in the culture system during genetic transduction of mammalian HSC. The first of these references, Hanenberg *et al.* (1996), teaches successful transduction of murine high-proliferative-potential colony forming cells sorted from bone marrow, and $CD34^+$-enriched human bone marrow or cord blood cells. The protocol included culturing the cells for 1 day in a pre-stimulation medium supplemented with IL-6 (100 units/mL) and stem cell factor (SCF; 100 ng/mL), followed by a 24-hour co-culture with retrovirus-containing supernatant on fibronectin fragments. The reference concludes that fibronectin provides for co-localization of the retrovirus and target cells on specific fibronectin fragments thereby facilitating genetic transduction of the mammalian cells. This reference does not teach or suggest the use of a combination of an mlp ligand and a flt3 ligand as part of the cytokine cocktail. Both of these factors are a requisite limitation in Applicants' claimed invention.

The second of the Group VI references, Hanenberg *et al.* (1997), is directed to optimization of the fibronectin-transduction protocol for human $CD34^+$ hematopoietic cells. This reference teaches culturing of $CD34^+$ BM, CB, and peripheral blood (PB) cells, $CD34^+CD38^+$ and $CD34^+CD38^-$ PB cells, or unseparated mononuclear cells. The cells were pre-stimulated for 1 to 2 days in stromal medium with a cytokine cocktail including various combinations of SCF (100 ng/mL, IL-6 (200 units/mL), G-CSF (100-200 ng/mL), and megakaryocyte growth and development factor (MGDF, i.e., mpl ligand) (100-200 ng/mL). Primary cells were then cultured on fibronectin fragment-coated plates in the presence or absence of one or more of these cytokines and transduced with retroviral-containing cell-free supernatant. The reference teaches that pre-stimulation of the cells with SCF alone or in combination with other cytokine(s) for 1-2 days prior to exposure to retrovirus improved

transduction efficiency, and that the optimal transduction efficiency occurred with the combination of SCF, G-CSF, and MGDF (page 2200,Table 1). This reference does not teach or suggest the use of a combination of an mlp ligand and a flt3 ligand as part of the cytokine cocktail. Both of these factors are a requisite limitation in Applicants' claimed invention.

Further, in both of the Group VI references, gene transfer efficiency was monitored 12-16 days following exposure of the $CD34^+$ hematopoietic cells to retroviral infection, and long-term engraftment capabilities of the transduced cells was not assessed. One of skill in the art would recognize that while these references suggest the advantages of fibronectin for enhancing transduction efficiency of cultured hematopoietic stem cells *in vitro,* they are a mere invitation to experiment further, as use of this protocol for *ex vivo* genetic manipulation of quiescent human hematopoietic stem cells would not necessarily yield a transduced population of HSC capable of supporting long-term engraftment. The state of the art at the time of Applicants' invention was replete with statements as to the difficulty of obtaining transduced multi-lineage pluripotent human hematopoietic stem cells as noted further herein below. Applicants respectfully note that an invitation to experiment is not sufficient grounds to reject an invention as obvious.

Unless the Examiner shows that at the time the invention was made there was something more than a mere suggestion of success, obviousness has not been demonstrated. Judge Rich explains in *In re Tomlinson* : "[t]here is usually an element of 'obviousness to try' in any research endeavor, that it is not undertaken with complete blindness, but with some semblance of a chance of success, and that patentability determinations [of obviousness] based on that as a test would not only be contrary to statute but result in a marked deterioration of the patent system as an incentive to invest in those efforts and attempts which go by the name of research." *In re Tomlinson* 363 F.2d 928, 931 (CCPA 1966).

In summary, the Group V and VI references teach successful transduction protocols for human $CD34^+$ hematopoietic stem cells. There is no teaching or suggestion of Applicants' combination of factors, i.e., fibronectin, an mpl ligand, and a flt3 ligand alone or in combination with the other cytokines recited in the pending claims. At most the Group VI references teach

that transduction of CD $34^+$ hematopoietic stem cells pre-cultured for 1-2 days with SCF, G-CSF, and MGDF and then cultured in the presence of fibronectin, SCF, G-CSF, and MGDF is beneficial for transduction efficiency of human CD34+ hematopoietic stem cells cultured *in vitro*.

Further, the Hanenberg *et al.* (1997) reference teaches an optimized transduction protocol for human $CD34^+$ hematopoietic stem cells that provides for successful transduction of these cells. Where successful results are taught in a scientific field replete with failures, there is no motivation to modify the reference or to seek guidance from other references in the art as to how the successful protocol can be modified to arrive at another successful way of achieving a particular objective. Of all the other factors taught by the Group I-V references, the Examiner has failed to provide the evidence for motivation within the Group V or VI references, or within the knowledge of one of skill in the art, to modify the teachings of those references, or to combine those teachings with the teachings of the Group I-IV references, to arrive at Applicants' claimed invention.

*The Group I, III, and IV References Directed to Culture Conditions for HSC Provide No Motivation to Combine the Cited References.*

The Examiner has relied on the Group I, III, and IV references to provide missing key components of Applicants' claimed transduction protocol not taught by the Group V and VI references. However, an analysis of the Group I III, and IV references reveals that Applicants' claimed invention is not rendered obvious by the combination of the teachings of these references with that which is taught by the Group V and VI references.

Group I References.

References within Group I are directed to methods of isolating and culturing populations of human hematopoietic stem cells. These references teach various combinations of factors to be included in a culture system, which combinations do not encompass the combinations of factors

set forth in Applicants' claimed invention. As the Examiner has noted, these references are not directed to transduction protocols; the Examiner has relied on the Group V and VI references to provide these elements of Applicants' claimed invention.

The first of these Group I references, Murray *et al.*, U.S. Patent No. 5,665,557, teaches methods of obtaining compositions enriched in human hematopoietic stem cells or hematopoietic megakaryocyte progenitor cells, and compositions comprising stem cells and populations of cells obtained by these methods. The methods comprise the use of a monoclonal antibody specific for the marker CDw109, whereby a subpopulation of human hematopoietic stem cells expressing this marker can be separated out from a population of human hematopoietic stem cells by screening for those cells specifically bound by this antibody. The Murray *et al.* patent teaches successful culture of a population of isolated stem cells expressing this marker in a medium containing IL-3 (10 ng/mL), GM-CSF (2 ng/mL), steel factor (SF; 100 ng/mL), and erythropoietin (EPO; 2 units/mL). See this patent at column 13, lines 16-20, and Table 5. Thus, the Murray *et al.* patent teaches a culture medium comprising 2 of the optional elements of the culture medium that serves as the basis of the presently claimed invention, i.e., IL-3 and steel factor (c-kit ligand). This patent does not teach or suggest the use of an mpl ligand (i.e., thrombopoietin, TPO, or a thrombopoietin mimetic) in combination with a flt3 ligand (FL) to culture multi-lineage pluripotent hematopoietic stem cells, which are both required elements in Applicants' claimed invention.

Murray *et al.* suggest that their culture methods provide a population of HSC that would be suitable for gene therapy application (column 10, lines 4-46), though this patent provides little guidance as to what transduction protocol should be followed. This patent thus provides an invitation to experiment with transduction of HSC cultured in the manner set forth in this patent specification.

Applicants respectfully note that one of skill in the art would not have been motivated to look to the other cited references for guidance as to additional elements to combine with the culture medium disclosed and/or claimed in the Murray *et al.* reference, as the culture medium taught in this patent is suitable for the intended objective, i.e., culturing a specific population of

hematopoietic stem cells bearing the CDw109 marker and which are suitable for gene therapy protocols. At most, one of skill in the art would look to the field of HSC gene transfer technology for guidance as to how to transduce CDw109-bearing HSC cultured in the manner described in the Murray *et al.* patent. Even if this inquiry led one of skill in the art to combine the teachings of the other cited references that are directed to gene transduction of HSC (i.e., Group V and VI references), Applicants respectfully submit that the end result would not be Applicants' claimed invention, as there would have been no motivation to modify the culture conditions identified by Murray *et al.* as providing excellent culture of pluripotent HSC.

The second of these Group I references, Nakahata *et al.*, U.S. Patent No. 5,861,315, teaches methods for culturing a population of human multipotential cells to obtain mature blood cells. The methods comprise culturing isolated human hematopoeitic progenitor and/or stem cells in a culture medium comprising a combination of stem cell factor (SCF), IL-6, and soluble IL-6 receptor. Thus this patent teaches use of 2 of the optional elements of the culture medium that serves as the basis of the presently claimed invention, i.e., IL-6, which the Nakahata *et al.* patent teaches at a concentration of 50 ng/mL, and SCF, a c-kit ligand, which the Nakahata *et al.* patent teaches at a concentration of 100 ng/mL (column 6, line 38). This patent does not teach or suggest the use of an mpl ligand (more particularly thrombopoietin, TPO, or a thrombopoietin mimetic) in combination with a flt3 ligand to culture human hematopoietic stem cells, which are both required elements in the stem cell culture protocols recited in Applicants' claimed invention.

Applicants respectfully note that one of skill in the art would not have been motivated to look to the other cited references for guidance as to additional elements to include in the culture medium taught by the Nakahata *et al.* patent for use in the methods of the Nakahata *et al.* patent, as the culture medium taught in this patent is identified as being the most suitable for the objective in question, i.e., culturing human hematopoietic stem cells to obtain mature erythrocytes. In contrast, Applicants provide a combination of factors that are to be present during culture and transduction of human HSC such that the pluripotent HSC proliferate, thereby

enhancing retroviral transduction of these proliferating HSC, without becoming progenitor cells committed to a particular pathway of lineage development. This objective is counter to that of the Nakahata *et al.* patent. Hence, Applicants respectfully submit that there was no motivation to modify the Nakahata *et al.* patent, or to combine the teachings of this reference with the teachings of the other cited references, to arrive at Applicants' claimed invention.

The third of these Group I references, Hoffman *et al.*, U.S. Patent No. 5,744,361, is directed to a method for culturing human hematopoietic stem cells that allows for serum-free culture in the absence of stromal cells and expansion of progenitor cells. The method comprises culturing the isolated cells in a culture medium that includes c-kit ligand alone or in combination with at least one of IL-3, GM-CSF, G-CSF, IL-1, IL-6, or a fusion protein of IL-3 with IL-1alpha, IL-3 with IL-6, or IL-3 with GM-CSF. Thus this patent teaches use of up to 3 of the optional elements of the culture medium recited in Applicants' claimed invention, i.e., c-kit ligand, which Hoffman *et al.* teaches at a concentration of 10 ng/mL to 500 ng/mL; and/or IL-3, which Hoffman *et al.* teaches at a concentration of 500 pg/mL-2 ng/mL; and and/or IL-6, which Hoffman *et al.* teaches at a concentration of 500 pg/mL-10 ng/mL. The Hoffman *et al.* patent also teaches that the most preferable combinations include c-kit ligand in combination with IL-3 and GM-CSF, where the IL-3 is a critical element that must be present in the culture medium. This is to be contrasted with Applicants' method of stem cell culture, where IL-3 is taught as an optional element. As with references 1 and 2 of Group I, the Hoffman *et al.* patent fails to teach or suggest the use of an mpl ligand (more particularly thrombopoietin, TPO, or a thrombopoietin mimetic) in combination with a flt3 ligand to culture hematopoietic stem cells, which are both required elements in the stem cell culture/transduction methods recited in Applicants' claimed invention.

Applicants respectfully note that one of skill in the art would not have been motivated to look to the other cited references for guidance as to additional elements to include in the culture medium taught by the Hoffman *et al.* patent for use in the methods of the Hoffman *et al.* patent, as the culture media taught in this patent are identified as being the most suitable for the

objective in question, i.e., serum-free culture of human hematopoietic stem cells in the absence of stromal cells to provide for expansion of progenitor cells. Hence, Applicants respectfully submit that there was no motivation to modify this reference, or to combine the teachings of this reference with the teachings of other cited references, to arrive at Applicants' claimed invention.

The fourth of these Group I references, Fei *et al.*, U.S. Patent No. 5,635,387, is directed to methods for increasing the number of human hematopoietic precursor cells *in vitro*, and provides a device for carrying out these methods. The methods comprise the steps of separating out human hematopoietic precursor cells from a blood product, inoculating the separated hematopoietic precursor cells into a suitable culture vessel containing a culture medium comprising a nutritive medium supplemented with a source of growth factors, and optionally, human or other animal plasma or serum, or microcarrier beads to which the cells are capable of attaching; the culture medium is absent bone marrow stromal elements. The Fei *et al.* patent teaches that suitable growth factors to be added to the nutritive medium include interleukins 1-15, erythropoietin (EPO), stem cell factor (SCF, i.e., c-kit ligand), granulocyte-clolony stimulating factor (G-CSF), granulocyte macrophase-colony stimulating factor (GM-CSF), macrophage-cology stimualating factor (M-CSF), tumor growth factor-β (TGF-β), tumor necrosis factor-α (TNF-α), the interferons, fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), and insulin-like growth factors. Preferred are combinations of growth factors, especially the combination of SCF, IL-1α, IL-3, and IL-6, at a concentration of between 0.5 and 100 ng/mL. See Fei *et al.* at column 9, lines 10-42. As with the other 3 references noted above, the Fei *et al.* patent makes no mention of including an mlp ligand or flt3 ligand within the culture medium described as successfully achieving proliferation of human hematopoietic precursor cells *in vitro.* These two factors are a necessary limitation in the culture medium used in Applicants' claimed method of genetically modifying human hematopoietic stem cells.

Again, one of skill in the art would not have been motivated to look to the other cited references for guidance as to additional elements to include in the culture medium taught by the Fei *et al.* patent for use in the methods of the Fei *et al.* patent, as the culture media taught in this

patent are identified as being the most suitable for the objective in question, i.e., culturing of human HSC to increase the number of these precursor cells *in vitro*. Hence, Applicants respectfully submit that there was no motivation to modify this reference, or to combine the teachings of this reference with the teachings of the other cited references to arrive at Applicants' claimed invention.

The fifth of these Group I references, Davis *et al.*, U.S. Patent No. 5,599,703, teaches an *in vitro* culture system that supports proliferation and amplification/expansion of both primitive hematopoietic bone marrow blood stem cells and $CD34^+$ progenitor cells for all hematopoietic cell lineages. Endothelial cells treated with cytokines serve as the basis of the culture system. The Davis *et al.* patent teaches that the use of endothelial cells as a support system provides an advantage over the use of bone marrow stromal cell monolayers because they are a homogeneous cell line that allows for prolonged culture (as many as 6 months) of these stem cells in the presence of low serum culture conditions (at column 8, lines 39-55). The Davis *et al.* patent teaches a method of culture that comprises contacting isolated human bone marrow hematopoietic $CD34^+$ stem and progenitor cells with endothelial cells, and then culturing the combination of these cells in the presence of at least one cytokine in an amount sufficient to support amplification/expansion of the hematopoietic $CD34^+$ stem and progenitor cells. The list of cytokines to be used is GM-CSF alone; GM-CSF + SCF; IL-3 + SCF + IL-6; and GM-CSF+IL-3 + SCF + IL-6. As noted by the Examiner, the Davis *et al.* patent teaches that the effective concentrations for these agents is 0.1-20 ng/mL for GM-CSF, 1.0-200.0 ng/mL for IL-3, 5.0-500 ng/ml for SCF, and 1.0-100 ng/mL for IL-6 (at column 9, lines 11-32). This patent further teaches that in the absence of the endothelial cell layer, the combinations of either GM-CSF + SCF, IL-3 + SCF + IL-6, or GM-CSF + IL-3 + SCF + IL-6 in liquid suspension culture failed to provide for amplification/expansion or maintenance of primitive $CD34^+$ cell number.

In view of these findings, the Davis *et al.* patent concludes that these growth factors alone are not responsible for the success of their *in vitro* culture system. Rather, this patent suggests that an interaction between the $CD34^+$ hematopoietic stem cells and the endothelial cells, other

soluble growth factors, membrane-bound growth factors, cellular adhesion molecules, or extracellular matrix proteins produced by cytokine-activated endothelial cells may be involved in the success of their culture system. See the Davis *et al.* patent at column 9, lines 34-52. Though this reference suggests other factors may be necessary to ensure maintenance of $CD34^+$ hematopoietic stem cells/$CD34^+$ progenitor cells, the patent clearly teaches that these factors can be provided by culturing the primitive CD34+ stem cells in contact with cytokine-treated endothelial cells. No where does this reference teach or even suggest that other growth factors or substances, more particularly the two elements, i.e, mpl ligand and flt3 ligand , that are required in the culture system set forth in Applicants' claimed methods of genetically modifying human stem cells, could or should be included in a cell culture system to replace the need for an endothelial cell layer in their claimed culture system.

Davis *et al.* suggest that their culture methods provide a population of HSC that would be suitable for gene therapy application (column 5, lines 8-13), though this patent provides no guidance as to what transduction protocol should be followed. This patent thus provides an invitation to experiment with transduction of HSC cultured in the manner set forth in this patent specification.

Applicants respectfully note that one of skill in the art would not have been motivated to look to the other cited references for guidance as to additional elements to combine with the culture medium disclosed and/or claimed in the Davis *et al.* reference, as the culture medium taught in this patent is suitable for the intended objective, i.e., i.e., culturing of human hematopoietic $CD34^+$ stem cells and $CD34^+$ progenitor cells to amplify/expand the number of these cells *in vitro*. At most, one of skill in the art would look to the field of HSC gene transfer technology for guidance as to how to transduce these HSC cultured in the manner described in the Davis *et al.* patent. Even if this inquiry led one of skill in the art to combine the teachings of the other cited references that teach gene transduction of HSC (i.e., Group V and VI references), Applicants respectfully submit that the end result would not be Applicants' claimed invention, as there would have been no motivation to modify the culture conditions identified by Davis *et al.*

as providing excellent culture of human hematopoietic $CD34^+$ stem cells and $CD34^+$ progenitor cells.

In summary, the references provided in Group I do not teach or suggest the use of an mpl ligand with a flt3 ligand to culture hematopoietic stems cells characterized by the capability of self-renewal and the ability to give rise to all hematopoietic cell lineages, and which provide for successful long-term engraftment following their genetic modification. Further, they do not teach or suggest the use of these two factors in combination with the other factors recited in Applicants' claimed invention, nor do they teach or suggest a method for genetically modifying human hematopoietic stem cells cultured in this manner by using retroviral vectors, adenoviral vectors, and adeno-associated viral vectors as the gene transfer vehicle. Further, because each of these references provides a successful means by which hematopoietic stem cells can be cultured, there was no motivation to modify any of these references, or to combine the teachings of these references with the other cited references, to arrive at Applicants' claimed invention.

Group III and IV References.

The Group III and IV references are relied upon to teach the role of LIF and the combination of SCF (c-kit ligand) and IL-6 in HSC function. They are not directed to genetic modification of human HSC. Applicants note that these references also provide no motivation for their modification, or for their combination with the teachings in the Group I, V, and VI references, to arrive at Applicants' claimed invention.

The first of the Group III references, Escary *et al.* (1993), describes the role of LIF in LIF-deficient mice derived by gene targeting techniques. This reference teaches that LIF-deficient mice have dramatically decreased numbers of stem cells in spleen and bone marrow, which can be restored by administering exogenous LIF from a mini-osmotic pump. The reference further teaches that injection of spleen and marrow cells from LIF-deficient mice promotes long-term survival of lethally radiated wild-type mice. The reference concludes that LIF in the microenvironment *in vivo* supports stem cell survival and/or renewal of HSC *in vivo*.

Thus, Escary *et al.* address *in vivo* supplementation of LIF to assist in reconstitution of the hematopoietic cell pool. This reference does not suggest or address the role of LIF in transduction protocols for HSC, nor does it provide the motivation to combine the teachings of this reference with the teachings of the other cited references to arrive at Applicants' claimed invention.

The second of the Group III references, Szilvassy *et al.* (1996), is directed to an investigation of the effects of LIF on maintenance of long-term repopulating murine stem cells when cultured on one of three murine stromal cell lines. Isolated primitive murine HSC with competitive long-term repopulating ability were co-cultured on the various murine stromal cell lines for 2 weeks in the presence or absence of LIF (10 ng/mL) and then assayed for cobblestone area-forming cell development. In some experiments IL-1β, IL-3, IL-6, macrophage colony-stimulating factor (M-CSF), GM-CSF, steel factor (SLF), or G-CSF were added (see the reference at page 4619, column 2). Szilvassy *et al.* teach that culturing the murine cells in the presence of LIF facilitated maintenance of the stem cell population numbers on one stromal cell line by inducing production of IL-6 and SLF by the stromal cells. The reference concludes that LIF plays an important role in maintaining repopulating stem cells *in vivo* by indirectly promoting IL-6 and SLF expression by bone marrow stroma (at page 4619, last paragraph of column 2). In arriving at this conclusion, Szilvassy *et al.* further state that HSC could be sustained *in vitro* on relatively simple stroma that have been molecularly engineered to express the appropriate spectrum of cytokines and/or extracellular matrix components that promote self-renewal *in vivo.* Thus, Szilvassy *et al.* provide the motivation to culture HSC in stromal cell lines supplemented with LIF or engineered to express LIF and other relevant factors involved in HSC function. This is not an invitation to modify this reference or to combine this reference with the teachings of the other cited references to arrive at Applicants' claimed invention. The protocol taught by Szilvassy *et al.*, which has as a requisite step the culturing of HSC on stromal cell lines, is a protocol designed to study HSC function, not to provide for transduction of HSC. Though this type of protocol can allow for further experimentation to elucidate the mechanisms that regulate HSC self-renewal versus a commitment to a specific hematopoietic lineage, it does

not provide the specific motivation to modify this reference, or to combine this reference with the other cited references, to arrive at Applicants' claimed invention.

The single Group IV reference, Bodine *et al.* (1992), assesses the effects of SCF on primitive murine hematopoietic cells *in vitro* and *in vivo*. Long-term repopulating murine stem cells were cultured in the presence of IL-3 (200 units/mL) alone, stem cell factor (SCF;100 ng/mL) alone, either of these in combination with IL-6 (100 ng/mL), or in the presence of all three. Though the reference demonstrates that limited numbers of long-term repopulating stem cells survive in the presence of SCF alone (page 918, second paragraph of Discussion), the reference concludes that the overall long-term repopulating ability of cells cultured in the presence of SCF, IL-3, and IL-6 was superior, about 3-fold greater that the repopulating ability of cells cultured in any combination of two factors (see, for example, at page 915, Figure 1, and page 918, third paragraph of Discussion). This reference does not suggest that other combinations of factors would provide better results, nor does it suggest which combination of factors would be best for genetically modifying HSC. Where this reference teaches a beneficial combination of factors for culturing murine HSC, Applicants respectfully submit that there is no motivation to modify the Bodine *et al.* reference, or to look to the other cited references for guidance as to how the Bodine *et al.* reference should be modified, to arrive at Applicants' claimed invention.

In summary, the teachings set forth in the Group III and Group IV references do not provide specific motivation to incorporate the teachings therein with the teachings of the Group I references, or with the additional teachings of the Group V and VI references, to arrive at Applicants' claimed invention.

*The Group II References Provide No Motivation to Combine the Cited References or At Most Provide an Invitation to Experiment in an Unpredictable Scientific Endeavor.*

The Examiner has further relied on the teachings of the Group II references to provide the teachings and/or suggestions to modify the Group I, III, IV, V, and/or VI references to arrive at Applicants' claimed invention. References within Group II are also directed to methods of culturing populations of murine or human hematopoietic stem cells in the presence of various growth factors.

The first of the Group II references, Ku *et al.* (1996), investigates the role of soluble thrombopoietin receptor (sTPOR) on culture of murine multipotential progenitors in the presence of EPO and one or more cytokines selected from steel factor (SF; 100 ng/mL), flt3 ligand (FL; 1 μg/mL), IL-6 (100 ng/mL), IL-7 (200 units/mL), and IL-11 ( 100 ng/mL) (see, for example, Tables 1, 2, 4, and 5). In one experiment, primitive HSC are cultured with EPO in the presence of sTPOR, TPO (200 ng/mL), or SF alone, or in the presence of sTPOR + TPO, sTPOR + SF, TPO + SF, or sTPOR + TPO + SF (Table 1, page 4126). Results of this particular study demonstrate that a combination of sTPOR and SF supported colony formation including formation of multilineage colonies that was equal to that supported by a combination of TPO and SF (at page 4126, Table 1 and column 1, lines 26-30). Ku *et al.* conclude that sTPOR shows a direct positive stimulation of the proliferation of primitive progenitors, despite the fact that this receptor partially blocks the stimulatory effects of TPO on megakaryocyte colony formation (see Figure 1). However, this reference fails to teach culture of human multi-lineage pluripotent stem cells in the presence of an mpl ligand and a ckit ligand, more particularly, in combination with fibronectin, to achieve Applicants' claimed method of genetic modification of this type of hematopoietic cells.

In summary, Ku *et al.* teach combinations of factors that are not part of Applicants' claimed invention and which provide for successful culture of murine multi-lineage pluripotent hematopoietic stem cells. Ku *et al.* do not suggest that their protocol should be modified, or combined with the teachings of the other cited references, to arrive at Applicants' claimed invention.

The second of the Group IV references, Ohmizono *et al.* (1996), reports on the results of a study designed to develop an effective culture system for expansion of human CB-derived CD34+ cells using various cytokines in combination with SCF (20 ng/mL) or flt3 ligand (20 ng/mL). The additional cytokines included IL-3 (10 ng/mL), IL-6 (100 units/mL), IL-11 (100 units/mL), and TPO (100 ng/mL). In all cases, the desired outcome is expansion of committed progenitors, and hence this reference does not report on the effects of the various cytokine cocktails on the ability of cultured CB-derived CD34+ hematopoietic stem cells to maintain their pluripotent state; the reference is not concerned with hematopoietic stem cell proliferation, rather, it is concerned with expansion of the stem cell population, and hence manipulation of these stem cells to commit to a particular hematopoietic cell lineage. Thus, the results of this study are not necessarily applicable to Applicants' claimed methods, as the particular combinations of factors used by Applicants' invention are designed to initiate division of the pool of quiescent pluripotent HSC to allow for proliferation of these stem cells so that gene transfer and integration can be maximized, and to minimize the amount of differentiation to committed progenitors and thus loss of pluripotency. Without analyzing the destiny of the population of multi-lineage pluripotent HSC, it is not feasible to predict the effects of these various combinations of cytokines on this population of cells. As such, the teachings of this reference do not provide the motivation to modify this reference, or to seek guidance from the other cited references as to how the protocols of Ohmizono *et al.* should be modified, to arrive at Applicants' claimed invention.

The third of the Group II references, Kobayashi *et al.* (1997), reports on an investigation of the effects of SF (100 ng/mL), flt3 ligand (FL; 100 ng/mL), and TPO (50 ng/mL) on the proliferation of primitive human bone marrow progenitors in serum-deprived culture supplemented with EPO. Other cytokines were optionally included in the culture system, including IL-3 (100 units/mL, IL-6 (100 ng/mL), and EPO (2 unit/mL). Human $CD34^+$ hematopoietic stem cells were enriched for primitive progenitors in the cell-cycle dormant state on the basis of the absence of the CD38 marker (i.e., $CD34^+CD38^-$ population), and assessed for clonal proliferation following 7

days of suspension culture in the presence of varying combinations of cytokines (page 428, column 1). The reference concludes that for this population of cells, no single factor was able to stimulate proliferation of these primitive progenitors. Of the two combination factors tested, SF + TPO or FL + TPO enhanced proliferation of cells more than the combination SF + FL. However, the greatest proliferation was seen with SF + FL + TPO. The reference concludes that SF + FL + TPO stimulates entry into the cell cycle of the majority of dormant progenitors, and addition of IL-3 to this combination did not induce proliferation of additional clones, though it increased the size of individual clones (Table 3, page 430, and column 1, lines 16-21, through column 2, line 2). However, this reference fails to suggest that this combination of factors would be applicable to a protocol for genetic modification of human hematopoietic cells, more particularly multi-lineage pluripotent HSC.

Further, as the Examiner has made of record in citing to the Group I-IV references, the state of the art at the time of Applicants' invention provided numerous cell culture protocols that were touted as being suitable for the intended objective of culturing human HSC. Applicants respectfully submit that there was not a motivation, suggestion, or teaching, either within the Kobayashi *et al.* reference, the other cited references, or within the knowledge of one of skill in the art, of the desirability of making the specific combination that was made by the Applicants to arrive at Applicants' claimed invention. As noted above, such specific motivation to combine references is required to render an invention obvious under 35 U.S.C. §103.

The fourth of the Group II references, Ramsfjell *et al.* (1997) investigates the effectiveness of TPO to stimulate stromal-independent growth, multi-lineage differentiation, and progenitor cell expansion from single primitive $CD34^{+}CD38^{-}$ human bone marrow cells cultured 12-14 days in serum-depleted liquid cultures. The reference teaches that TPO (50 ng/mL) alone stimulated limited clonal growth, but synergized with c-kit ligand (KL; 50 ng/mL), flt3 ligand (FL; 50 ng/mL), or IL-3 (50 ng/mL) to potently enhance clonogenic growth (Abstract; page

5171, column 2, first paragraph; Figure 1A, page 5171). This enhancement effect was increased further when the cells were cultured in the presence of TPO plus at least two other cytokines, for example, plus KL and FL; plus KL and IL-3; and plus KL and IL-3 and IL-6 in the presence or absence of EPO (page 5171, column 2, second and third paragraphs, and Figure 1A). The increase in clonal growth in the presence of TPO and multiple cytokines reflected not only an increase in number of colonies but also an increase in size of each colony. The synergistic enhancement effects of TPO were not as dramatic in $CD34^+CD38^+$ HSC, which represent more committed progenitors (see Figure 1B at page 5171). Thus, Ramsfjell *et al.* conclude that the ability of TPO to synergistically enhance progenitor cell growth in combination with other cytokines such as KL, FL, and IL-3 is restricted to the more primitive $CD34^+CD38^-$ progenitor cells (page 5172, column 1, lines 1-5).

Ramsfjell *et al.* also demonstrated that the $CD34^+CD38^-$ progenitor cells having increased growth potential in the presence of TPO in combination with KL and FL, or KL and IL-3 and IL-6, represented $CD34^+CD38^-$ progenitor cells with multi-lineage potential (Figure 4, page 5173; page 5173, column 2). Further, this reference teaches that TPO synergizes with a combination of KL + FL, or with a combination of KL + FL + IL-3 + IL-6 to stimulate prolonged production and expansion of CFC, including multipotent progenitor cells, from $CD34^+CD38^-$ progenitor cells (Figure 5, page 5174; column 1, third paragraph, page 5174). The Ramsfjell *et al.* reference cautions that it had not yet been demonstrated whether TPO or any other known cytokine might expand the true long-term reconstituting pluripotent stem cells (column 2, page 5176, lines 12-16). True to this statement, Ramsfjell *et al.* provide no data with respect to long-term engraftment potential of their cultured HSC.

The Ramsfjell *et al.* reference concludes that TPO and other early activating cytokines such as KL and FL, have clinical applications for expanding hematopoietic progenitor cells *ex vivo*, which could then be used in conjunction with high dose chemotherapy or in gene therapy protocols (page 5176, last paragraph of column 1, continuing through column 2). However, no guidance as to what gene therapy protocols should be followed is provided. Applicants respectfully submit that there was not a motivation, suggestion, or teaching, either within the

Ramsfjell *et al.* reference, the other cited references, or within the knowledge of one of skill in the art, of the desirability of making the specific combination that was made by the Applicants to arrive at Applicants' claimed invention. As noted above, such specific motivation to combine references is required to render an invention obvious under 35 U.S.C. §103.

Thus, the Group II references teach to varying degrees the synergistic role of TPO in combination with various early acting cytokines on continued proliferation and multi-lineage expansion of primitive $CD34^+CD38^-$ progenitor cells. Of these references, Ramsfjell *et al.* provide evidence of the synergistic role of TPO in combination with other cytokines, particularly the combination of KL + FL, or KL + FL + IL-3 + IL-6, on proliferation and multi-lineage expansion of primitive $CD34^+CD38^-$ progenitor cells, and further suggest the use of TPO in combination with these cytokines in *ex vivo* gene therapy protocols. However, this reference provides no guidance with respect to how such genetic manipulation should be carried out. As noted previously, an invitation to experiment is not sufficient legal grounds to render an invention obvious. Accordingly, Applicants respectfully submit that the teachings of the Group II references, alone or in combination with the teachings of the other cited references, do not provide the motivation, suggestion, or teaching of the desirability of making the specific combination that was made by Applicants to arrive at Applicants' claimed invention.

The Examiner contends that one of skill in the art apprised of the teachings in the Group II references would have been motivated to combine these teachings with the teachings of all of the other references cited to arrive at Applicants' claimed invention. However, Applicants contend that even if one of skill in the art would have been motivated to combine the teachings of the Group II references with those taught in the Group I, III, and IV references, the end result would not have been Applicants' claimed invention, as the Group I, III, and IV references do not provide the missing information as to what protocol should be used to successfully achieve genetic modification of human hematopoietic stem cells in a manner that provides for self-renewal of the genetically modified stem cells as well as the potential for expansion of

genetically modified multi-lineage progenitors, both of which are required for successful long-term engraftment of genetically modified HSC.

The Examiner has relied on the Group V and VI references to provide the missing elements of Applicants' claimed invention that could be combined with the teachings of the Group II references to arrive at Applicants' claimed invention. However, Applicants respectfully submit that one of skill in the art, having been apprised of the Group V and VI references, would have moved forward with one of the transduction protocols taught in those references, as each of these Group V and VI references teaches successful genetic modification of HSC in a scientific field replete with references as to the difficulty in obtaining genetic modification of human pluripotent HSC.

Applicants urge the Examiner to carefully consider the state of the art with respect to genetic modification of human HSC at the time of Applicants' invention. Accordingly, a review of the references cited in Group V and VI, reveals that the prior art was in agreement as to the difficulty of gene transduction of the stem cell pool thought to serve as the source of multi-lineage pluripotent HSC necessary for successful long-term engraftment, i.e., those stem cells capable of self-renewal as well as proliferation of multipotent progenitors. Thus, for example, Fletcher *et al.* (1991) teach that "[a] major limiting factor in the development of retroviral vector-mediated gene transfer for treatment of diseases affecting the blood and blood-forming tissues has been the low infection efficiency of hematopoietic stem cells in vitro (page 837, column 1, lines 1-4). Similarly, Hanenberg *et al.* (1996) state that "[u]se of gene transfer technology in the human trials has been hampered by the low frequency of gene delivery to the hematopoietic stem cell targets" (page 876, column 1, lines 11-13), and "clinical trials using this approach [cell-free virus containing supernatant in the infection protocol] have demonstrated levels of gene transfer into repopulating hematopoietic stem cells that appear to be too low for considering gene therapy as a treatment option" (page 876, column 1, last line, through column 2, line 3); and quoting a report on National Institutes of Health investment in research on gene therapy, "significant problems remain in all basic aspects of gene therapy. Major difficulties at the basic level include shortcomings in all current gene transfer vectors and an inadequate understanding of the

biological interaction of these vectors with the host" (Hanenberg *et al.* (1996), at page 879, column 2, under Discussion section). Again, Hanenberg *et al.* (1997) point to the difficulty in HSC genetic modification, stating:

> Although initial studies in mice demonstrated that retroviral vectors based on the murine Moloney leukemia virus can infect long-term reconstituting murine stem cells . . . clinically applicable protocols in humans . . . and in large animal models . . . have been limited because of the low gene transfer efficiency achieved. These low levels of gene transfer can be attributed to both characteristics of the target cell population as well as the biology of the retroviral vectors. (Hanenber *et al.* (1997), page 2193, second column, through page 2194, column 1, line 8).

Thus, at the time of Applicants' invention, the skilled artisan was well aware of the difficulty in obtaining genetically modified human HSC. Applicants contend that one of skill in the art, even apprised of the combinations of factors identified in the Group I-IV references, would not have been motivated to modify the teachings of the Group V and VI references, which all taught successful gene transduction of stem cells, to arrive at Applicants' claimed invention. Nor would one of skill in the art have been motivated to combine the teachings of the Group V and VI references with the teachings set forth in the Group I-IV references, none of which demonstrate successful genetic modification of pluripotent human hematopoietic stem cells, to arrive at Applicants' claimed invention. Where successful gene transduction protocols are taught in a field replete with unpredictable results, there is no motivation to modify those successful protocols, or to combine the teachings of the other cited references with those of the successful transduction protocols, to arrive at Applicants' claimed invention. Furthermore, even if one of skill in the art had been motivated to combine the teachings of all of these references, one of skill in the art would not have believed that there was a reasonable expectation of success in view of the well-recognized problems associated with inefficiency of gene transduction of human pluripotent HSC at the time of Applicants' invention.

In view of these remarks, Applicants maintain that the requisite showing of motivation to combine the Group I-VI references that is required for a rejection under 35 U.S.C. §103(a) has

not been demonstrated in the instant case. Therefore, the rejection of the claims under 35 U.S.C. §103(a) should be withdrawn.

## CONCLUSION

In view of the aforementioned amendments and remarks, Applicants respectfully submit that the rejection of the claims under 35 U.S.C. §103(a) is overcome and should be withdrawn. Applicants submit that this application is now in condition for allowance. Early notice to this effect is solicited. If in the opinion of the Examiner a telephone conference would expedite the prosecution of the application, the Examiner is invited to call the undersigned.

It is not believed that extensions of time or fees for net addition of claims are required, beyond those that may otherwise be provided for in documents accompanying this paper. However, in the event that additional extensions of time are necessary to allow consideration of this paper, such extensions are hereby petitioned under 37 C.F.R § 1.136(a), and any fee required therefore (including fees for net addition of claims) is hereby authorized to be charged to Deposit Account No. 16-0605.

Respectfully submitted,

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Polly P. Burton

Polly P. Burton

**Version with Markings to Show Changes Made:**

Please amend claims 20, 24-27, 31, 37, 39-42, and 51 to read as follows:

20. (Three times amended) The method according [t]to claim 19, further comprising culturing the hematopoietic stem cells in the presence of interleukin 3 (IL-3) in a concentration range of about 5 ng/mL to about 200 ng/mL.